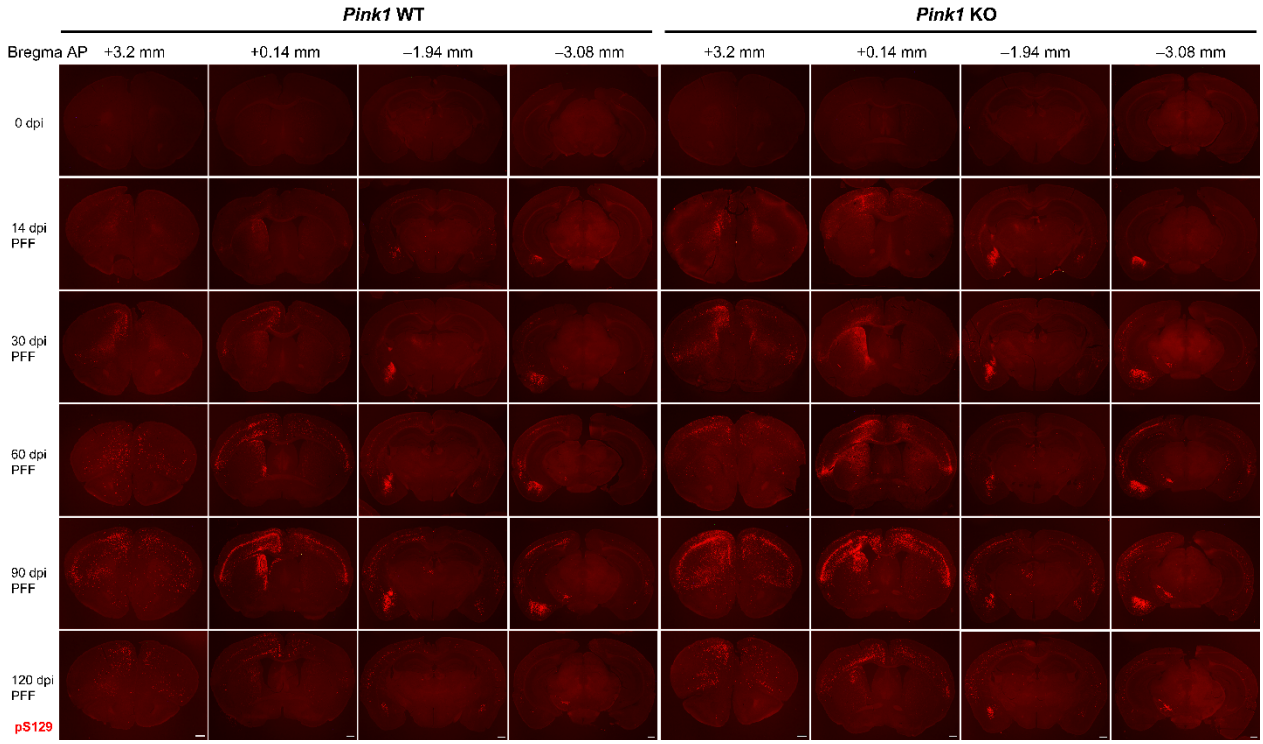
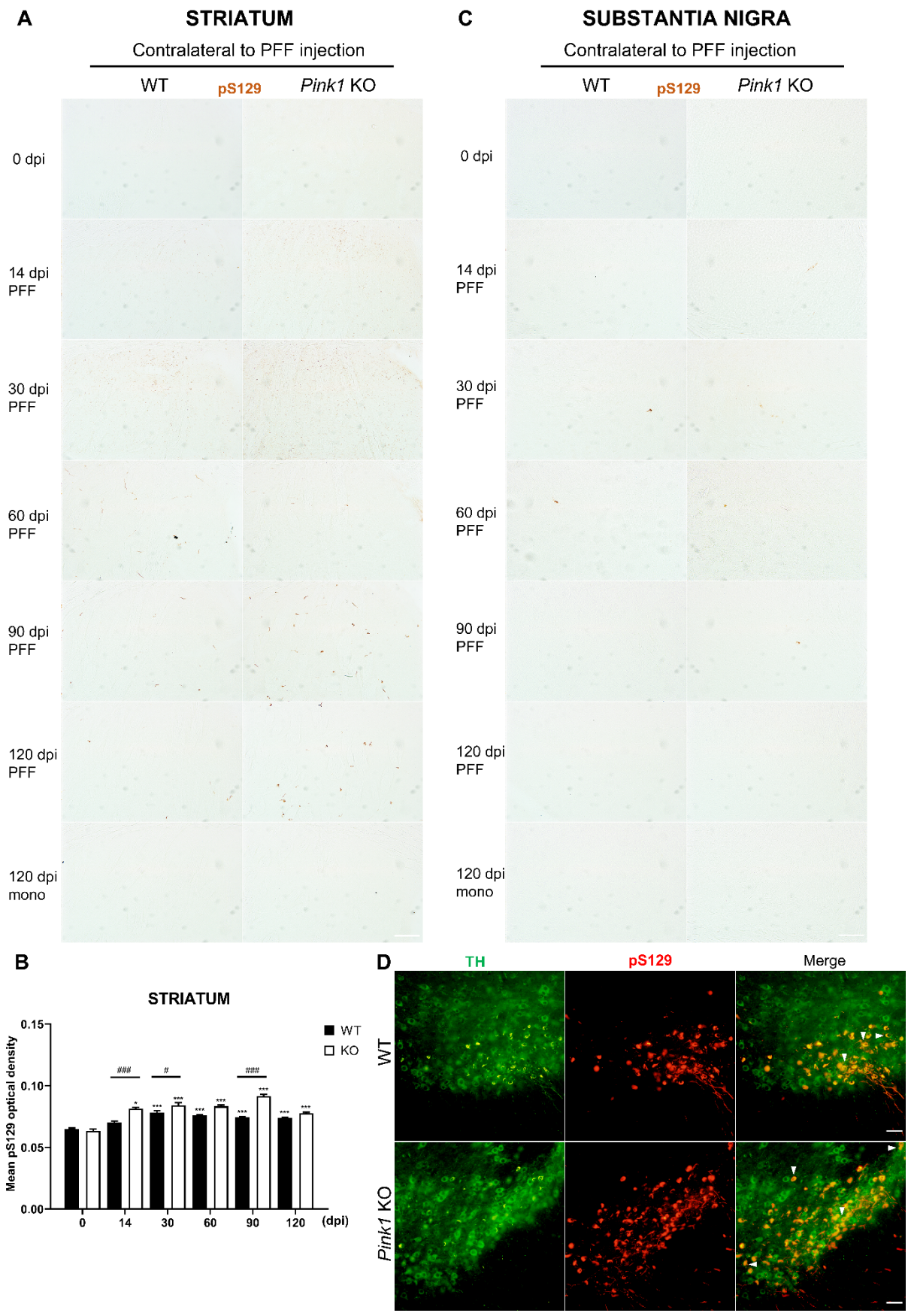
Supplementary Material

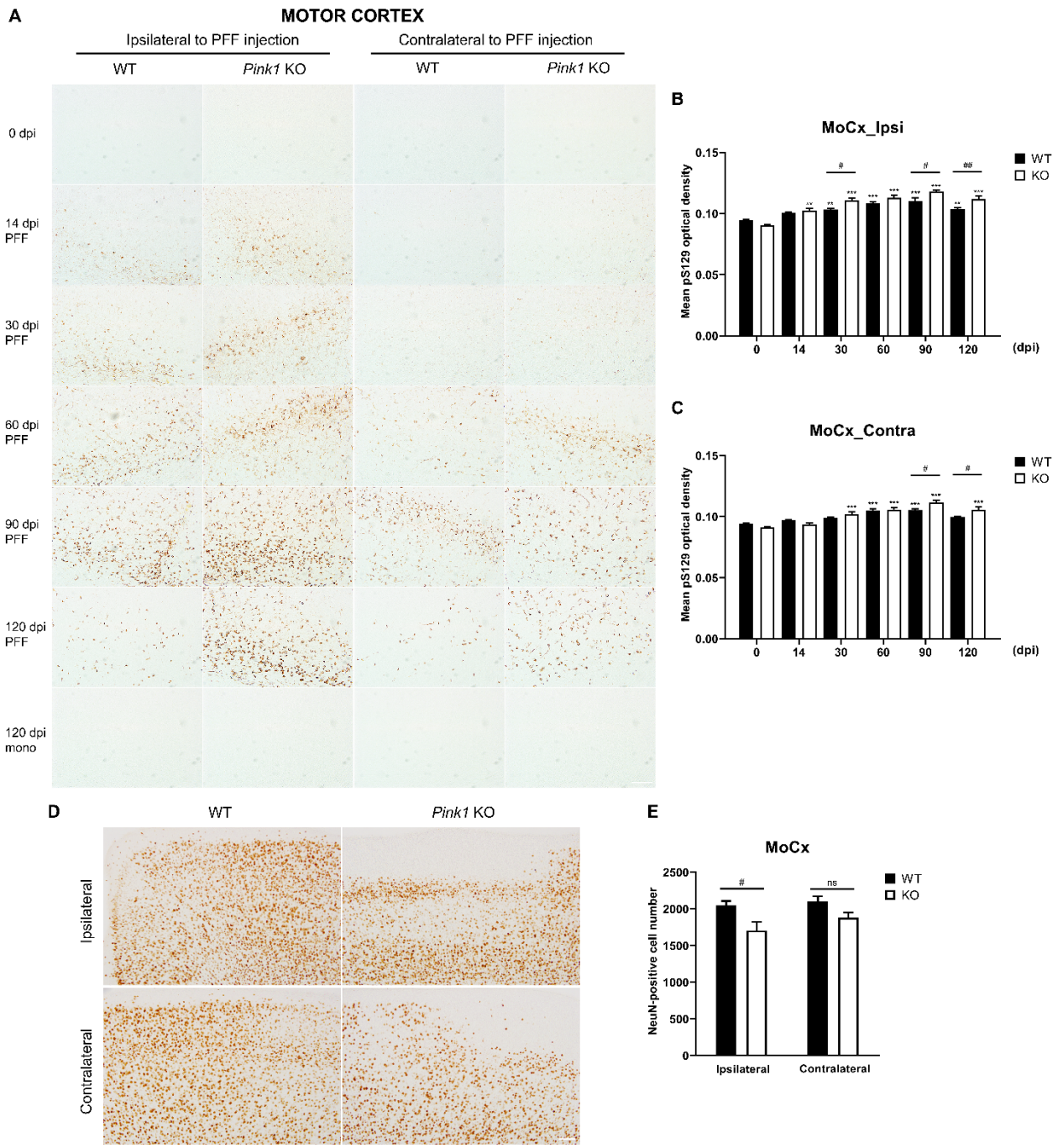
PTEN-Induced Putative Kinase 1 Dysfunction Accelerates Synucleinopathy



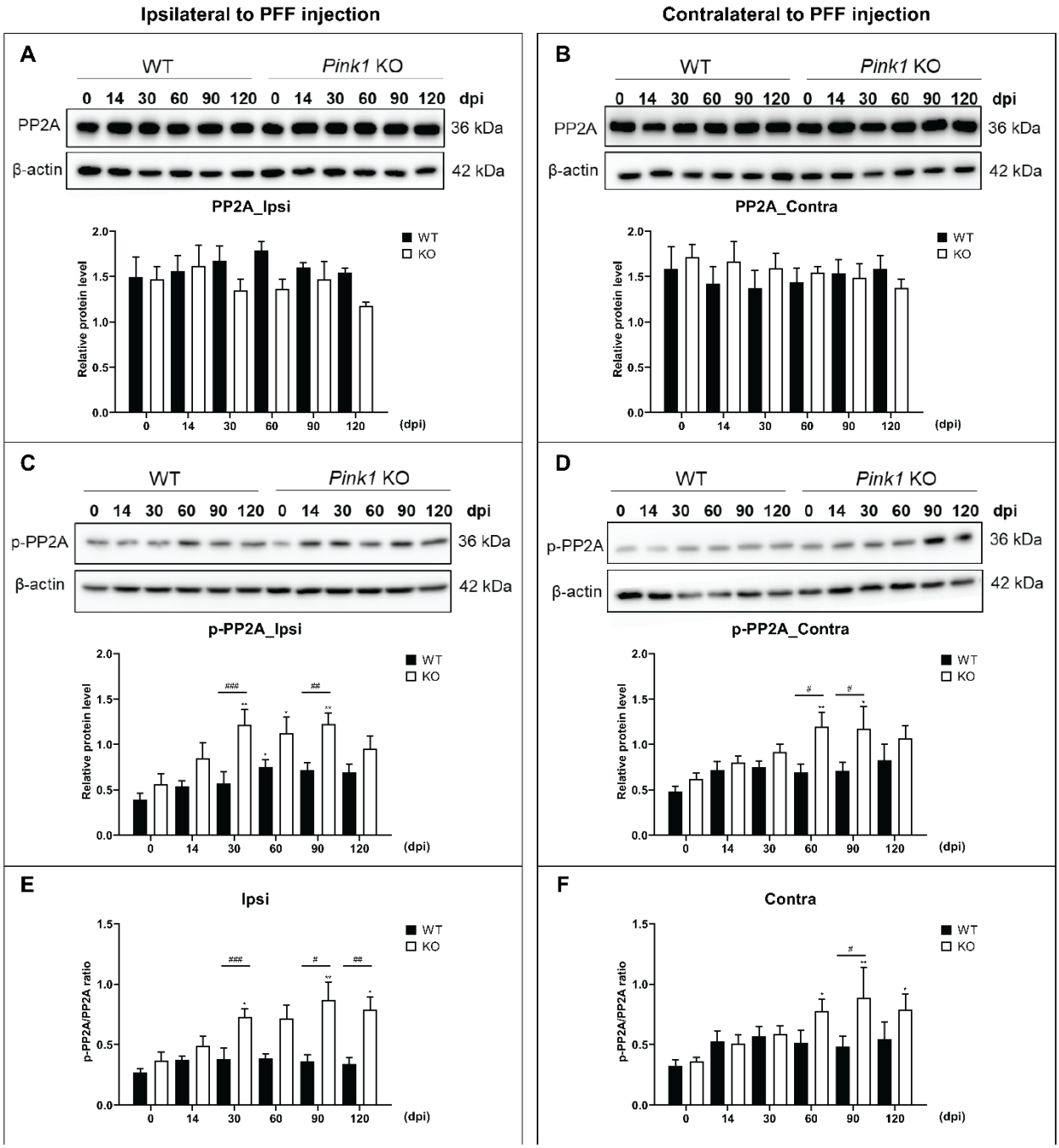
Supplementary Figure 1. Temporal accumulation of phosphorylated α-syn aggregates in WT and *Pink1* KO mice after intrastriatal PFF injection. Mouse brain tissues were stained with antibody against pS129-α-syn as red color. Coronal sections of mouse brain at approximately same levels coordinated to bregma at anterior–posterior (A–P) of +3.2 mm, +0.14 mm, –1.94 mm, and –3.08 mm were showed in both groups (left side of each layer indicated the ipsilateral to PFF injection). Scale bar = 500 μm. dpi, day post-injection; PFF, α-syn preformed fibril; pS129, antibody detected phosphorylated at serine 129 α-syn; WT, wild type; KO, knockout.


A
STRIATUM
Contralateral to PFF injection
WT
pS129
Pink1 KO
0 dpi
14 dpi PFF
30 dpi PFF
60 dpi PFF
90 dpi PFF
120 dpi PFF
120 dpi mono
C
SUBSTANTIA NIGRA
Contralateral to PFF injection
WT
pS129
Pink1 KO
0 dpi
14 dpi PFF
30 dpi PFF
60 dpi PFF
90 dpi PFF
120 dpi PFF
120 dpi mono
B
STRIATUM
Mean pS129 optical density
0.15
0.10
0.05
0.00
WT
KO
###
#
###
0
14
30
60
90
120
(dpi)
D
TH
pS129
Merge
WT
Pink1 KO

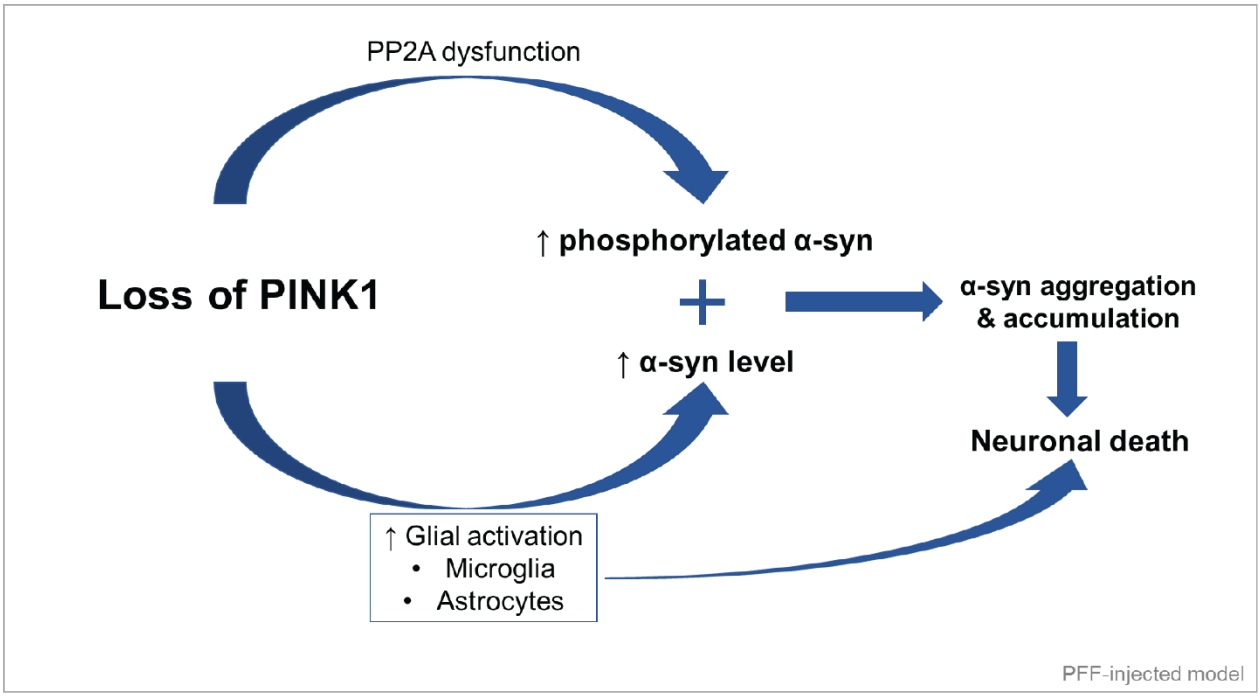
Supplementary Figure 2. Pathological α-syn in the contralateral striatum and substantia nigra. Representative images of pS129-α-syn aggregates with DAB staining after PFF or monomeric α-syn injection in the contralateral striatum (A) and its quantification (B). DAB staining of pS129-α-syn aggregates after PFF or monomeric α-syn injection in the contralateral substantia nigra (C). Scale bar = 100 μm. Lewy body- and Lewy neurite-like α-syn inclusions in dopaminergic neurons in the ipsilateral SN of WT and *Pink1* KO mice at 3mpi (D). White arrowhead showed co-localization of pS129-α-syn within dopaminergic neurons. Scale bar = 100 μm. Statistical significance was determined using one-way *ANOVA* followed by post-hoc *Sidak's* test and two-way *ANOVA* followed by post-hoc *Turkey's* test for multiple group comparison. mono, monomeric α-syn; TH, tyrosine hydroxylase; Ipsi, ipsilateral; Contra, contralateral; otherwise are same as previous figures.



Supplementary Figure 3. Pathological α-syn and neurodegeneration in the motor cortex. Representative images of pS129-α-syn aggregates with DAB staining after PFF or monomeric α-syn injection in the MoCx (A). Scale bar = 100 µm. Quantification of pS129-α-syn optical density in the ipsilateral MoCx (A) and contralateral MoCx (C). Representative images showing NeuN-positive immunoreactivity (D) and the number of NeuN-positive cells per field of view (E) in the MoCx of *Pink1* KO and WT mice 120 days after PFF injection. Scale bar = 100 µm. Statistical significance was determined using one-way *ANOVA* followed by post-hoc *Sidak's* test and two-way *ANOVA* followed by post-hoc *Turkey's* test for multiple group comparison. MoCx, motor cortex; NeuN, neuronal nuclear protein; otherwise are same as previous figures.



Supplementary Figure 4. Increased phosphorylation of protein phosphatase 2A in PFF-injected *Pink1* KO mice. Representative immunoblots of PP2A expression level in the whole brain and its quantification analysis normalized to β-actin in the ipsilateral brain (A) and contralateral brain (B). Representative immunoblots of p-PP2A expression level in the whole brain and its quantification analysis normalized to β-actin in the ipsilateral brain (C) and contralateral brain (D). Changes in p-PP2A/PP2A ratio (E, F). Statistical significance was determined using one-way *ANOVA* followed by post-hoc *Sidak's* test and two-way *ANOVA* followed by post-hoc *Turkey's* test for multiple group comparisons. PP2A, protein phosphatase 2A; p-PP2A, phosphorylated protein phosphatase 2A at Tyr307; otherwise are same as previous figures.



Supplementary Figure 5. Processes might contribute in PFF-injected *Pink1* knockout mouse model. Loss of PINK1 result in PP2A dysfunction and accelerate microglial and astrocytic activation, consequently, increase α-syn aggregation, and accumulation. The glial hyperactivation might also lead to neuronal loss.